Low Budget iGem Project

TITLE: Inexpensive and simple Miniprep and electrophoresis for *E. coli*

Introduction

When doing a synthetic biology project, there are several protocols that must be followed to obtain the wanted results. This protocols can cost a lot of money and most of the time there aren't the necessary funds available to obtain the best materials and at the same time have the necessary parts to complete the project.

Our low budget proposal consists of two modified techniques: Miniprep and electrophoresis. Both techniques are widely important when doing a project and we chose them because we could prove our Miniprep results in an electrophoresis that is also low budget. When combining both protocols, the amount of money saved can be impressive.

Miniprep is a very common technique used to obtain plasmid DNA from bacteria. A Miniprep has three basic solutions that are what separates plasmid DNA from everything else that is inside the cell, by killing the bacteria in order to release the DNA, and then by purifying the plasmid DNA, taking advantage of its solubility in alcohol.

Electrophoresis is one of the most widely used procedures in the Biology fields for separating DNA fragments. The gel is submersed in a tank filled with a salted solution that allows the electric current to flow and separate the DNA fragments according to size. Small holes are punctured near the negative pole where the samples are loaded with a loading dye to help track the DNA migrating to the positive pole. Afterwards, the gel is introduced into ethidium bromide, which binds tightly to the DNA double helix, and makes it glow underneath ultraviolet light.

In this work, we will propose an alternate Miniprep method, consisting of three new recipes for the solutions used in this protocol made of low-cost everyday materials, without the loss of purity in the results. Just as well, we will present an inexpensive electrophoresis chamber design using a Tupperware, graphite electrodes, and an easy way to modify the alternative current from our laboratory to a direct current by means of a diode bridge.

Theoretical framework

As mentioned before, Miniprep contains three alkaline solutions that isolate plasmid DNA from everything else. The first solution consists of EDTA and Tris-HCl. This solution takes the pH to 8 with the Tris buffer and start weakening the cell membrane, preparing it for lysis. The second solution consists of NaOH and SDS, the last one is what makes lysis happen since it interferes with lipids and proteins in the cell membrane by degrading them, while NaOH denatures plasmid and chromosomal DNA. The third solution contains glacial acetic acid and potassium acetate. This solution neutralizes the pH, but most importantly, potassium acetate precipitates lipids, proteins, and chromosomal DNA, leaving plasmid DNA in the solution.

Part of our low budget proposal is changing the three solutions used in Miniprep without having a drastic change on the results; this involves DNA concentration, salts, proteins, chromosomal DNA, and RNA that are left with plasmid DNA. We found out that cilantro is a natural chelate agent and is a good replacement for EDTA, creating our new solution 1. Next, we used a commercial detergent called Axion to replace SDS in the second solution. Finally, we created a buffer that could neutralize the reactions occurring so plasmid DNA could be renatured in the solution. For this buffer we used sodium bicarbonate (baking soda) and lime.

On the other hand, electrophoresis works on the concept of a current flowing from a negative pole to a positive one, separating macromolecules based on size. In order to do so, an average 100 volts for an hour is needed. In the conventional procedure, an expensive high voltage power supply is used. In this work, we present a simple and cheap replacement that changes the laboratory's alternating current to direct current through means of a diode bridge. Thus, the electrophoresis will use 110 volts, which would separate the macromolecules in a shorter time.

One of the prime problems electrophoresis presents is the violent oxidation of the positive pole. For this reason, platinum-based electrodes are the best option for electrodes, however, they present a high cost. As a replacement, we suggest the use of graphite electrodes made out of HB pencils. Unlike steel and other metals, graphite isn't oxidized at room temperature, but at extreme heat. (Xiaowei, 2004)

Two of the most common components of the buffer and loading dye are Tris and EDTA, both of which are expensive. We decided to replace Tris with NaOH, another base, and EDTA with the same cilantro extract we will use in the Miniprep protocol. With this, we will propose a new and cheaper buffer and loading dye.

For the buffer, we will use SB Buffer, composed of boric acid and NaOH. Due to its low conductivity, DNA runs slowly in the SB buffer, taking up to 3 hours, instead of 1 hour. However, given the low conductivity, the bands look sharper and you could run the gel at a higher voltage without the risk of melting the gel. Also, we will add the cilantro extract to substitute the EDTA in the conventional TAE buffer.

Methodology

Solution 1	**30 ml**
Tris-HCl	0.1181 g
Cilantro extract	130 µl
Distilled water	29.87 ml

To obtain cilantro extract:

1. Weigh 3 grams of cilantro leaf for 30 ml of distilled water. (1:10)
2. Agitate at 80°C for 4 hours
3. Filter on Whatman filter #1
4. Centrifuge at 14,000 rpm for 5 minutes
5. Store at -20°C

Solution 2	**30 ml**
NaOH	24 g
Axion Detergent	3 ml
Distilled water	27 ml

Solution 3	**30 ml**
Sodium bicarbonate	5 g
Filtered lime	8 ml
Distilled water	22 ml

Miniprep Protocol

1. Leave an inoculum, depending on how many tests you want to make. (For each 20 ml you obtain 2 Eppendorf tubes with the culture)
2. Centrifuge the starter culture for 5 min at 3000 g
3. Decant supernatant and leave a little over 2 ml and resuspend
4. Pass 1 ml to an Eppendorf tube, and create as many tests as you wish
5. Centrifuge at 14,000 rpm for 1 minute
6. Decant supernatant
7. Add 200 µl of solution 1
8. Vortex until pellet is dissolved
9. Incubate for 5 minutes at 37°C
10. Add 200 µl of solution 2
11. Mix gently by inversion
12. Incubate 5 minutes at 37°C
13. Centrifuge at 14,000 rpm for 10 minutes
14. Pass 500 µl of supernatant to a new tube (be sure not to touch pellet)
15. Add 1 ml of EtOH 100% (-20°C)
16. Incubate at -20°C (10 minutes – 2 hours)
17. Centrifuge at 14,000 rpm for 10 minutes
18. Decant supernatant
19. Add 200 µl EtOH 70% (-20°C)
20. Centrifuge at 14,000 rpm for 5 minutes
21. Decant supernatant

22. Dry pellet at 37°C
23. Add 30 µl of H2O nuclease free
24. Resuspend with vortex
25. Incubate at 37°C for 20 minutes.
26. Measure DNA concentration at Nanodrop

Electrophoresis chamber, power supply, and electrodes:
Tupperware with lid
Diode bridge
2 crocodile clips
Duplex polarized cables 1 m
Plug with duplex polarized cables
4 wire connectors
2 pencils (HB)
Insulating tape

- Electrodes: For the electrodes, you can either use the graphite center of an HB pencil or buy a carbon electrode for soldering.
  Note: For this procedure, we used the graphite center of an HB pencil.
- Chamber: Take the Tupperware lid and puncture two holes, one for each electrode. Make sure they can fit through, and that they don't move around.
- Power supply:
  1. Add the four wire connectors to the diode bridge.
  2. Take the duplex polarized cables and peel the ends. Leave about an inch and a half of bare copper wiring.
  3. Pick the crocodile clips and insert the copper wiring on each one. Using a pair of pliers, press the ends of the crocodile clips so the copper wires are secured. Add insulating tape.
  4. Connect the duplex polarized cables to the positive and negative ends of the diode bridge. Using a pair of pliers, press the ends of the wire connectors to secure the duplex cables.
  5. Take the plug and connect its two cables to the remaining ends of the diode bridge, the ones corresponding to the alternative current. Using a pair of pliers, press the ends of the wire connectors to secure the plug.
  6. NOTE: If your laboratory uses 220-240 volts, you can add a step down converter to lower the current to a voltage of 100-120 volts.

Loading dye:

For the loading dye, we tried three recipes: the conventional one with Tris and EDTA and equal amounts of blue and red, one with NaOH and the cilantro extract and equal amounts of blue and red, and the last one with NaOH and the cilantro extract and five times the amount of red over blue.

Loading dye (Tris/EDTA)	1 ml	**Loading dye (NaOH)**	1 ml	**Loading dye (red)**	1 ml
10 mM Tris	100 µl (0.1M)	10 mM NaOH	100 µl (0.1N)	10 mM NaOH	100 µl (0.1N)
60 mM EDTA	200 µl (0.3M)	Cilantro extract	200 µl	Cilantro extract	200 µl
60% glycerol	600 µl	60% glycerol	600 µl	60% glycerol	600 µl
0.03% blue	18.6 µl (1.61%)	0.03% blue	18.6 µl (1.61%)	0.03% blue	18.6 µl (1.61%)
0.03% red	10.7 µl (2.8%)	0.03% red	10.7 µl (2.8%)	0.15% red	53.57 µl (2.8%)
Distilled water	70.7 µl	Distilled water	70.7 µl	Distilled water	27.83 µl

Electrophoresis:

SB buffer	1L 10x
Boric acid	22.5 g
NaOH	4 g
Distilled water	1L

1. To make 400 ml of the SB buffer 1X: Combine 5 ml of the cilantro extract, 40 ml of the SB buffer 10X, and the remaining amount of distilled water.
2. Add 0.4 g of agarose to a beaker with the 50 ml of SB buffer 1X.

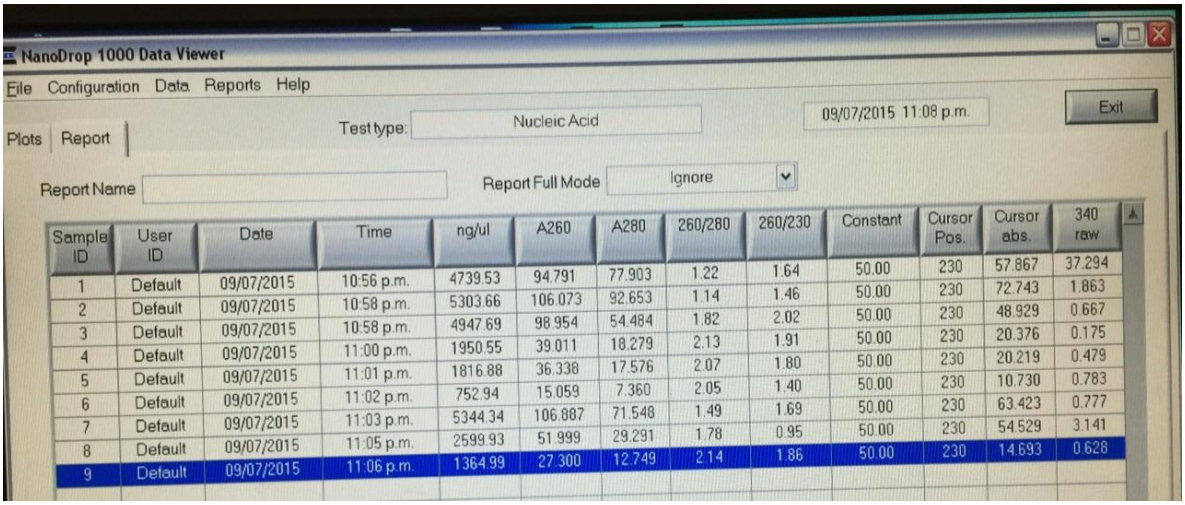
3. µwave until the agarose is fully melted and cool down for a few minutes. (µwave little by little to prevent the solution from rising out of the beaker)
4. Pour the agarose solution into the gel box. Carefully pop or shove to the side any bubbles (with the help of a pipette tip), put the comb in place, and let it cool for about 15-30 minutes until the gel solidifies (cover with aluminum foil to avoid contamination).
5. Place the gel inside the Tupperware.

Loading the gel

1. Fill the chamber with the rest of the SB buffer 1X (350 ml) and carefully add the agarose gel.
2. Mix 4 µl of the DNA sample with 1 µl of the loading dye.
3. Pour the mixture in the wells. Be careful with the bubbles and avoid breaking the gel with the pipette.
4. Place the lid and secure it.
5. Insert the electrodes and grasp them with the crocodile clips.
6. Plug it and run for 2-3 hours
7. To stain the gel, submerge it in ethidium bromide for half an hour.
8. Place it under ultraviolet light to visualize.

Results

Miniprep

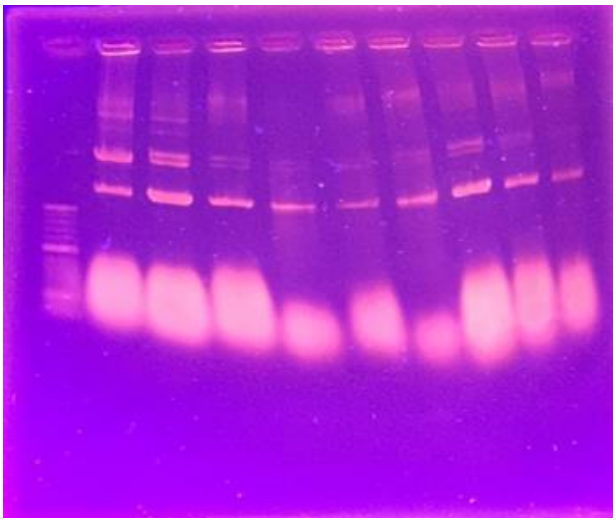


Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
1	Default	09/07/2015	10:56 p.m.	4739.53	94.791	77.903	1.22	1.64	50.00	230	57.867	37.294
2	Default	09/07/2015	10:58 p.m.	5303.66	106.073	92.653	1.14	1.46	50.00	230	72.743	1.863
3	Default	09/07/2015	10:58 p.m.	4947.69	98.954	54.484	1.82	2.02	50.00	230	48.929	0.667
4	Default	09/07/2015	11:00 p.m.	1950.55	39.011	18.279	2.13	1.91	50.00	230	20.376	0.175
5	Default	09/07/2015	11:01 p.m.	1816.88	36.338	17.576	2.07	1.80	50.00	230	20.219	0.479
6	Default	09/07/2015	11:02 p.m.	752.94	15.059	7.360	2.05	1.40	50.00	230	10.730	0.783
7	Default	09/07/2015	11:03 p.m.	5344.34	106.887	71.548	1.49	1.69	50.00	230	63.423	0.777
8	Default	09/07/2015	11:05 p.m.	2599.93	51.999	29.291	1.78	0.95	50.00	230	54.529	3.141
9	Default	09/07/2015	11:06 p.m.	1364.99	27.300	12.749	2.14	1.86	50.00	230	14.693	0.628

	Normal miniprep			Low Budget		
	Sol I	Sol II	Sol III	Sol I	Sol II	Sol III
1	X	X	X			
2		X	X	X		
3	X		X		X	
4	X	X				X
5	X				X	X
6		X		X		X
7			X	X	X	
8				X	X	X
9				X	X	X

Electrophoresis

The first electrophoresis we ran was with electrodes made from stainless steel skewers. However, the positive electrode suffered from intense oxidation due to the hydrolysis reaction present. The following tests were made with the graphite center of an HB pencil, which gave the following result:



Gel under ultraviolet light, ran with homemade electrophoresis chamber.

As seen in the photograph, the samples didn't come out as straight as they could have. This is due to the electrodes we used, if the table where the chamber is placed is subjected to constant movement, the electrodes will start shuffling around and the image will turn out skew.

Cost comparison

Miniprep

Normal Miniprep cost

Article	**Cost USD (per L or kg)**
Tris – HCl 1.5M	$28.00
EDTA (Tris-EDTA buffer solution)	$170.42
NaOH	$25.00
SDS	$347.00
Acetic Acid	$111.60
Sodium Acetate	$84.00
TOTAL	$766.02

Low Budget miniprep cost

Article	**Cost USD (per L or kg)**
Tris – HCl 1.5	$28.00
Cilantro	$1.50
NaOH	$25.00
Axion Detergent (750mL)	$1.12
Sodium bicarbonate	$3.64
Lime	$0.72
TOTAL	$59.98

Even though to create the three solutions is really cheap in both protocols, you can't buy the materials in small amounts, that's why all prices are also shown per liter or kg. The real struggle is buying the materials in large quantities since it's very expensive.

Electrophoresis

In the tables below, we present the cost of the materials and reagents we changed. The amount used is equivalent to that needed to make 1 L of buffer and the cost of the electrophoresis kit. The prices were taken from Sigma Aldrich, except for the chamber and the power supply, which were taken from Fischer Selective Products.

Electrophoresis	Amount	USD	Amount used	Price USD
Tris	500 g	99.24	48.4 g	9.606432
EDTA	100 g	22.07	3.7 g	0.81659
Glacial acetic acid	1000 ml	84.00	11.4 ml	0.9576
Chamber	1	634.10	1	634.1
Power supply	1	638.00	1	638
Platinum electrodes	1	169.92	2	339.84
			TOTAL	**1623.320622**

Low Budget	Amount	USD	Amount used	Price USD
NaOH	1000 ml	23.85	1 ml	0.02385
Cilantro	200 g	0.31	3 g	0.00465
Boric acid	500 g	45.98	22.5 g	2.0691
Tupper	1	3.06	1	3.06
Power supply	1	9.78	1	9.78
Graphite electrode	1	0.55	2	1.1
			TOTAL	**16.0376**

Discussion

After several trials, we found out that our procedure gave similar results to the normal Miniprep protocol. However, there were some trials in which we didn't obtain the expected results, so it's important to make new solutions once a week because this can interfere with the results. While doing the protocol, there were some visible differences in how the solutions reacted, but it's due to the nature of the components that we are using.

In electrophoresis, typically, platinum-based electrodes are used to avoid oxidation, but they present a high cost. We tried using graphite electrodes made from HB pencils and they proved to be suitable. The only disadvantage they present is the need to replace them after a few rounds, because they begin to fall apart. However, this doesn't present a problem for the gel, as the graphite doesn't stain it. Also, if the chamber is subjected to movement, the electrodes will slide, and the DNA samples won't come out straight. However, the separation of the bands of DNA in the gel is not affected by this, and the results can still be visualized.

Furthermore, the buffer suggested works perfectly; however, as it presents a lower conductivity, the electrophoresis has to run for a longer period of time (2 to 3 hours). Even though it works slowly, the DNA bands come out sharper than with the conventional TAE buffer. Just as with the buffer, we replaced the EDTA in our loading dye with cilantro extract and added blue and red coloring which resulted in a cheaper blue/red loading dye. We decided to use blue and red instead of the conventional blue and orange because it is easier to find a set of blue, green, yellow, and red colors. However, if you wish to use orange instead of red, the same percentage of coloring (0.15%) would be needed.

Conclusion

After many trials with our solutions and the normal solutions, we found out that you can mix normal Miniprep solutions and our solutions and get a good result. It's important to keep in mind that since we use natural resources such as cilantro and lime, you need to constantly prepare new solutions, preferably once every week, in order to maintain accurate results.

Obtaining the materials needed for a normal Miniprep protocol and electrophoresis can be a struggle for teams that don't have the necessary funds. By having an alternative, the difference in cost can be inverted in the project and obtaining materials and parts that are much more expensive. Our low budget Miniprep is almost 13 times cheaper than buying the usual Miniprep solutions (considering 1L and 1kg material), while our electrophoresis chamber and preparation is more than a 100 times cheaper. Even though the Miniprep results may vary, it's a real cost benefit for teams, and with time, there can be more modifications to create the most effective low budget Miniprep protocol. Meanwhile, the electrophoresis's only constraint is the construction of the power supply and the preparation of solutions, it's also important to remember that in this procedure the gel has to be run for a longer time.

Miniprep and electrophoresis are two of the most widely used procedures in Synthetic Biology. In this work, we offered alternatives for both, presenting alternatives that are both cheaper and safer than the conventional ones. We are sure that these modifications can help other teams save money and invest it on essential parts for their project. However, there is still room for other modifications to have even better results, with more accuracy and precision.

References

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